Original Article

Early-Life Stress and the Development of Obesity and Insulin Resistance in Juvenile Bonnet Macaques

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Stress is a risk factor for chronic illnesses such as obesity, type 2 diabetes, and hypertension and has been postulated to cause the metabolic syndrome via perturbation of the hypothalamo-pituitary-adrenal (HPA) axis. In our model of early-life stress (variable foraging demand [VFD]), food insecurity is imposed on monkey mothers for 16 weeks beginning when their nursing offspring are 3–5 months of age. Under VFD, food availability is never restricted, and the infant's growth is unaffected. VFD rearing does, however, cause a range of neurobiological abnormalities, including dysregulation of the HPA axis, manifested in abnormal cerebrospinal fluid cortisol and corticotropin-releasing factor levels. We previously reported spontaneous occurrence of metabolic syndrome in 14% of normally reared peripubertal bonnet macaques given ad libitum access to standard monkey chow. Here, we show that compared with normally reared monkeys, peripubertal VFD juveniles exhibit greater weight, BMI, abdominal circumference, and glucagon-like peptide-1 and decreased glucose disposal rates during hyperinsulinemic-euglycemic clamps. Our data suggest that early-life stress during a critical period of neuro development can result in the peripubertal emergence of obesity and insulin resistance. *Diabetes* 56:1382–1386, 2007

Stress is a risk factor for chronic illnesses such as obesity, type 2 diabetes, and hypertension (1) and has been hypothesized as a key element in the fetal origins of these adult diseases (2). Perturbation of the hypothalamo-pituitary-adrenal (HPA) axis, the body's central stress modulating mechanism, has been postulated to cause the metabolic syndrome (1). In studies of early-life stress, Rosenblum and Smiley (3) developed an experimental procedure in which mothers of nursing monkeys are exposed to circumstances in which the effort to obtain food varies between periods of easy access and relative difficulty, termed variable foraging demand (VFD). VFD is imposed for only 16 weeks, beginning when offspring are 3–5 months of age, and does not result in food deprivation or altered growth in mother or infant.

VFD rearing results in offspring with psychological sequelae similar to patients with anxiety and mood disorders, such as blunted affect, diminished capacity for affiliative engagement, and social subordinance (4), as well as persistent shifts in the basal and response-dependent levels of HPA axis and other neuroendocrine and immunological factors, such as cerebrospinal fluid (csf) corticotrophin-releasing factor (CRF) and transforming growth factor-β (5–9). We have also shown an enduring effect of VFD rearing on magnetic resonance spectroscopy imaging measures considered reflective of neuronal integrity and metabolism in brain regions implicated in trauma-related psychiatric disorders (10).

We have previously demonstrated a peripubertal, juvenile-onset metabolic syndrome using modified clinical criteria in normally reared bonnet macaques fed a life-long ad libitum diet of standard monkey chow (11). The prevalence of this syndrome in the colony is similar to that found in human populations (12) and may reflect genetic traits recently described in baboons (13) and the "unnatural" constant availability of food provided in laboratory settings. Compared with normal subjects, adult and peripubertal monkeys with the metabolic syndrome exhibited significantly greater BMI, insulin levels, insulin-to-glucose ratio (IGR), homeostasis model assessment (HOMA), and triglycerides (TGs). In humans, food insecurity has been shown to affect various physical, psychological, and social aspects of development (14). We hypothesized that early-life stress, in the form of maternal food insecurity during nursing, may predispose offspring to obesity and insulin resistance by the time of puberty onset.

RESEARCH DESIGN AND METHODS

The colony consists of ~250 bonnet macaques (*Macaca radiata*), born and reared in a social, seminaturalistic environment (11). Our monkeys begin eating solids around 2 months of age, with weaning beginning at 7–8 months. Regardless of rearing condition, monkeys are separated from their mothers and socially housed with peers at the end of the 1st year of life. Males reach puberty at ~4–4.5 and females at 3–3.5 years; full maturity is attained at 7–8 years of age. The control rearing condition entails ad libitum access to a commercial laboratory diet (Monkey Diet 5038; LabDiet, Richmond, IN; 4 kcal/g, 69% carbohydrate, 18% protein, and 13% fat), regularly supplemented with fresh fruit. All monkeys are treated ethically, under careful veterinary supervision in accordance with the State University of New York–Downstate Medical Center–Institutional Animal Care and Use Committee and Association

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CRF, corticotrophin-releasing factor; CRL, crown-rump length; csf, cerebrospinal fluid; GLP-1, glucagon-like peptide-1; HFD, high foraging demand; HOMA, homeostasis model assessment; HPA, hypothalamo-pituitary-adrenal; IGR, insulin-to-glucose ratio; LFD, low foraging demand; TG, triglyceride; VFD, variable foraging demand.

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TABLE 1
Metabolic and morphometric parameters in 48 differentially reared juvenile bonnet macaques aged 3–4 years

	VFD	Control	*P* value
n	30	18	
Weight (kg)	3.9 ± 0.07	3.6 ± 0.1	0.009
BMI (kg/m^2)	22.8 ± 0.34	21 ± 0.44	0.003
Abdominal circumference (cm)	28.0 ± 0.47	26.3 ± 0.61	0.034
Insulin (pmol/l)	56.6 ± 7.98	63.2 ± 11.64	NS
Glucose (mg/dl)	61.8 ± 1.79	64.3 ± 1.34	NS
IGR	0.84 ± 0.14	0.85 ± 0.09	NS
HOMA	141.8 ± 31.19	163.5 ± 20.45	NS
TG (mg/dl)	20.2 ± 1.79	20.5 ± 1.34	NS
HDL (mg/dl)	50.6 ± 2.06	50.2 ± 2.66	NS
VLDL (mg/dl)	3.5 ± 0.25	2.4 ± 0.33	0.009
GLP-1 (pmol/l)	95.4 ± 11.4	51 ± 15.9	0.029
Ghrelin (pg/ml)	2711 ± 212	2765 ± 289	NS
Leptin (pmol/l)*	51.4 ± 20.82	75.1 ± 22.54	NS
Adiponectin (μg/ml)	14.7 ± 1.19	17 ± 1.54	NS
C-peptide (pmol/l)	288.2 ± 27.67	223.4 ± 35.78	NS

Data are means ± SE. *P* values from ANCOVA covaried for age and sex. *Leptin values were detected in only seven VFD and six control animals.

for Assessment and Accreditation of Laboratory Animal Care and National Institutes of Health guidelines.

The primary sample consisted of 48 peripubertal bonnet monkeys (30 VFD and 18 controls; 25 males and 23 females; aged 3–4 years). A subset of 15 male macaques matched for weight and BMI (8 VFD and 7 controls) underwent euglycemic-hyperinsulinemic clamp studies.

VFD rearing. Mother-infant dyads are randomly assigned to control or treatment rearing conditions shortly after birth. Beginning when their infants are ~3–5 months old, mothers of the VFD-reared subjects confront ~4 months of circumstances in which the time and effort required to obtain food are either relatively brief and easy (low foraging demand [LFD]; essentially ad libitum access) or more lengthy and difficult (high foraging demand [HFD]). Alternating periods of LFD and HFD in 2-week blocks is termed VFD. Animals always have ample food, and frequent weight and health checks confirm normal growth and development in all subjects. After the experimental period and at the time of testing, all offspring are on ad libitum feeding. Further details of the rearing procedure are provided by Rosenblum and Smiley (3).

There were no differences between the randomly selected mothers undergoing VFD versus the controls with respect to parity or phenotypic characteristics related to body size, insulin resistance, fat distribution, obesity, or blood lipids (D.K., M.A.B., I.S., E.L.P.S., J.D.C., L.A.R., J.G.K., unpublished data). Furthermore, baseline levels of plasma and csf cortisol and CRF were similar in both groups of mothers. Similarly, there were no recorded phenotypic differences between mothers with different timing of the VFD experience ("early" vs. "late" postpartum) (15).

Blood chemistry. After an overnight fast (food was withdrawn at 1600 h, while water remained available ad libitum), between 0800 and 1100 h, monkeys were individually captured in carrying cages, placed in single-animal squeeze cages, and anesthetized with ketamine (10–15 mg/kg). Venous blood was drawn from antecubital or femoral veins and was immediately placed on ice, centrifuged for plasma separation, and stored at −80°C within 1 h in plain nonheparinized tubes, as described previously (11). Samples drawn during clamp studies were handled similarly. Samples were analyzed by routine laboratory procedures for glucose; TGs; and total, HDL, and LDL cholesterol at the University Hospital of Brooklyn Clinical Chemistry Laboratory (Brooklyn, NY). Insulin, C-peptide, glucagon-like peptide-1 (GLP-1), adiponectin, leptin, and ghrelin levels were analyzed using the Human Endocrine Lincoplex kit (Linco Diagnostic Services). As simple measures of insulin resistance, IGR and the HOMA (16) were calculated.

Morphometry. During anesthesia for blood sampling, weight in kilograms, crown-rump length (CRL), and abdominal circumference were measured. CRL is the length in centimeters from the vertex of the head to the base of the tail. Abdominal circumference is the largest distance in centimeters around the abdomen at the level of the iliac crests. Measurements were performed by the same team of investigators blinded to rearing condition. A modified BMI was calculated as mass in kilograms divided by the square of the CRL in meters (17).

Clamps. All euglycemic-hyperinsulinemic clamp studies were conducted by a team of two trained investigators at 0900 h after a 17-h period of food deprivation following procedures described by Bodkin et al. (18) in rhesus monkeys. In the afternoon before the day of the clamp, monkeys were captured in carrying cages and transferred to individual squeeze cages for overnight fasting. To decrease the stress of dehabituation and social isolation, experimental animals were kept in a pen along with other similarly aged individually caged monkeys with food in their cages. To reduce muscle fasciculation associated with ketamine, a muscle relaxant, xylazine, was added to the anesthesia. On the morning of the procedure, an intramuscular injection of a 9:1 mix of ketamine (100 mg/ml) and xylazine (20 mg/ml) solutions was used for induction of anesthesia and readministered as needed to achieve complete sedation throughout the procedure. The monkey was placed in a neonatal incubator with an ambient controlled temperature of 25–30°C. Heart rate, sphygmomanometric blood pressure, and core temperature measurements were continuously monitored. Bilateral antecubital or saphenous veins were cannulated, with one line used for infusates and the other for blood sampling. To maintain line patency, 1 cc heparinized saline (500 units heparin sodium in 60 cc 0.9% normal saline) was infused in the sampling line with each blood draw.

After baseline fasting plasma glucose levels were taken, a priming infusion of porcine insulin was administered followed by a continuous infusion (>40 mU/m^2 body surface area per min) for 120 min. Body surface area was calculated using a modified DuBois formula (19). Simultaneously, a variable-rate solution of 20% glucose was infused to maintain a euglycemic state (~85 mg/dl) determined by blood samples (0.05–0.1 cc) taken every 5 min from the sampling line, centrifuged for plasma separation, and analyzed by a Beckman Glucometer 2 (Beckman Instruments, Brea, CA). Glucose disposal is determined as the mean glucose infusion rate in milligrams per kilogram per minute during the 0- to 30-, 30- to 60-, 60- to 90-, and 90- to 120-min intervals (*M* rates). An additional 3 cc blood was drawn at 0, 30, 60, 90, and 120 min for a related study. Upon completion of the procedure, the cannulas were removed, and the animal was placed in a specially prepared cage and allowed to recover completely. Monkeys were returned to their home pens in late afternoon or early morning.

Statistical analysis. Means ± SE are reported throughout. Sex distribution comparisons used χ^2 analysis. Simple between-group comparisons used two-tailed Student's *t* tests, ANCOVA covaried for age and sex, as indicated, and repeated-measures ANCOVA covaried for age. Partial correlations were controlled for age. Not all monkeys had values for every parameter examined. Missing data led to exclusion on a measure-by-measure basis. Outlier values (>3 SD from the mean) for weight and BMI in one VFD female; for GLP-1 in one VFD female; for insulin, IGR, and HOMA in one VFD female; and for glucose, IGR, HOMA, and TGs in another control female led to exclusion from respective ANCOVAs and correlations. All statistical analyses were made using SPSS for Windows (release 10.0.1; SPSS, Chicago, IL) with $P < 0.05$ as the criterion for significance.

RESULTS

Primary sample. VFD juveniles had significantly greater weight [$F_{(1,43)} = 7.42$; $P = 0.009$], BMI [$F_{(1,43)} = 10.25$; $P =$

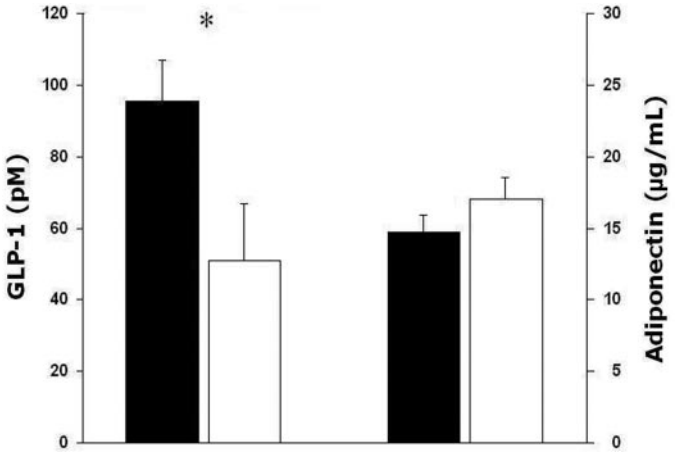

FIG. 1. Comparisons of mean ± SE GLP-1 and adiponectin levels in VFD (■) versus controls (□). *$P < 0.03$.

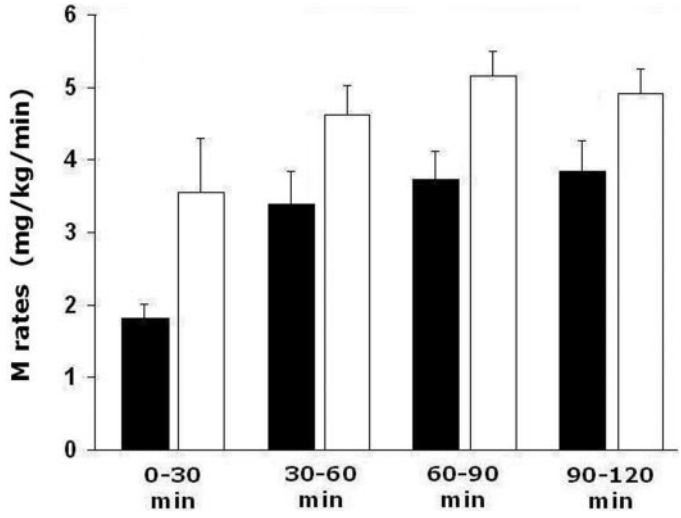

FIG. 2. Mean ± SE glucose disposal (M rates) during hyperinsulinemic-euglycemic clamps in eight VFD (■) versus seven control (□) male juvenile monkeys. Repeated-measure ANCOVA covaried for age; $F_{(1,12)} = 5.5$; $P = 0.037$.

0.003], abdominal circumference [$F_{(1,44)} = 4.81$; $P = 0.034$], and VLDL [$F_{(1,44)} = 7.37$; $P = 0.009$] than controls when covaried for age and sex (Table 1). No rearing group differences were noted for insulin, C-peptide, glucose, IGR, HOMA, adiponectin, ghrelin, or leptin. Leptin values were detected in only seven VFD and six control animals. GLP-1 levels were greater in VFD juveniles [$F_{(1,40)} = 5.14$; $P = 0.029$; Fig. 1], with no sex or sex by rearing group interactions. GLP-1 levels were inversely related to BMI ($r = -0.49$; $P = 0.011$).

Females across both rearing groups had higher levels of insulin [$F_{(1,31)} = 5.32$; $P = 0.028$], IGR [$F_{(1,30)} = 4.65$; $P = 0.039$], HOMA [$F_{(1,30)} = 4.22$; $P = 0.049$], and TGs [$F_{(1,44)} = 12.32$; $P = 0.001$] but weighed less than males [$F_{(1,44)} = 10.81$; $P = 0.002$] when covaried for age. Females also had greater adiponectin levels [$F_{(1,45)} = 6.15$; $P = 0.017$], with no rearing or sex by rearing interactions and had marginally higher C-peptide [$F_{(1,45)} = 3.8$; $P = 0.058$] and lower ghrelin levels [$F_{(1,37)} = 4.06$; $P = 0.051$; data not shown]. HDL, LDL, and total cholesterol did not differ by sex or rearing group status.

Blood pressure during anesthesia was highly variable and did not exhibit any statistically significant differences, consistent with our findings across ages, sex, rearing conditions, and methods of anesthesia (ketamine alone vs. ketamine plus xylazine) and of blood pressure measurement.

Clamps. Among the male monkeys who underwent clamps, no differences in age-adjusted fasting plasma glucose measures were noted between control and VFD juveniles [VFD = 83.8 ± 2.74 vs. control = 83.2 ± 2.74; $F_{(1,11)} = 0.018$; $P = 0.896$]. Similarly, no rearing group differences were noted for fasting insulin, IGR, or HOMA (all $P > 0.4$). VFD *M* rates, however, were lower at all 30-min clamp time intervals, with an overall significant difference between VFD and controls [repeated-measures ANCOVA; $F_{(1,12)} = 5.5$; $P = 0.037$; Fig. 2]. Additionally, across both rearing groups, BMI was negatively correlated with *M* rates at the 0- to 30-min ($r = -0.69$; $P = 0.003$), 30- to 60-min ($r = -0.51$; $P = 0.044$), 60- to 90-min ($r = -0.72$; $P = 0.002$), and 90- to 120-min ($r = -0.66$; $P = 0.006$) intervals, whereas GLP-1 was negatively correlated with *M* rates at the 30- to 60-min ($r = -0.68$; $P = 0.007$), 60- to 90-min ($r = -0.59$; $P = 0.026$), and 90- to 120-min ($r = -0.55$; $P = 0.042$) intervals.

DISCUSSION

Several experimental models demonstrate distinct relationships between disturbances of early rearing and persistent behavioral alterations (20,21), modulation of neuroendocrine and immunological systems (22–24), and permanent changes in neuroanatomical architecture (25). To our knowledge, this is the first study to report differences in metabolic parameters relating to obesity and insulin resistance in differentially reared nonhuman primates.

VFD rearing resulted in higher weight, BMI, and abdominal circumferences. In light of the chronic behavioral and neuroendocrinological effects of VFD rearing, further studies of consumption parameters and their relationship to measures of central stress may determine whether increased ingestion of "comfort food" (26) led to this difference when compared with controls. Strong negative correlations between BMI and *M* rates demonstrate the close clinical association between obesity and insulin resistance in this species. As studies of obese adult rhesus macaques of Hansen and Bodkin (27) have shown, pancreatic β-cell hyperresponsiveness is the earliest stage in the progression of insulin resistance to overt diabetes. In our colony, hyperinsulinemia is present in juvenile monkeys with the metabolic syndrome (11), yet VFD juveniles, although more obese than controls, did not have different levels of insulin, glucose, IGR, or HOMA. They did, however, exhibit lower *M* rates by hyperinsulinemic-euglycemic clamps. Unlike simple indicators, the clamp procedure measures in vivo glucose disposal as an index of whole-body tissue sensitivity to insulin.

GLP-1, a "brain-gut" peptide (28), is the most potent intestinal incretin hormone, which has been shown to enhance pancreatic β-cell maturation and proliferation, in fact promoting glucose disposal (28,29). In VFD juveniles in our stress-related model of insulin resistance, early increases in GLP-1 with robust negative correlations with *M* rates imply that circulating GLP-1 is an early marker of stress, contemporaneous with adrenergic inhibition of insulin secretion. Longitudinal studies are required to ascertain whether GLP-1 participates in a neuro-adaptive response involved in the pathogenesis of type 2 diabetes.

Consistent with our previous experience, compared

with males, peripubertal females showed general trends toward insulin resistance and the metabolic syndrome, including higher insulin, IGR, HOMA, and TGs, despite weighing less. This could be due to the earlier appearance of puberty in females of this species (30), although it remains to be determined whether the puberty-related insulin resistance normally seen in humans (31) is present in our macaques.

Adiponectin, an adipose-specific peptide with activity in the brain (32), was elevated in the early stages and decreased in the later stages of progression to insulin resistance and diabetes in adult rhesus macaques (33). As with the increased GLP-1 levels in VFD juveniles, elevations of this peptide with clear protective anti-atherogenic properties and strong correlations with insulin sensitivity (33), may represent a compensatory response to the earliest emergence of insulin resistance in peripubertal females.

Most experimental models of obesity and insulin resistance use gestational and postnatal nutritional and endocrinological manipulations (34–36). These studies underscore the importance of both plasticity and vulnerability of susceptible neural circuits in establishing and defending adult body weight and energy homeostasis (37). To our knowledge, ours is the only model of stress-related insulin resistance in juvenile nonhuman primates, without conventional factors that lead to obesity, such as dietary manipulation or restricted physical activity. The early decreases of in vivo insulin action may suggest that maternal food insecurity during nursing, a perceived threat to nutrition serving as an allostatic load (38), may trigger in the offspring an incretin response enabling glucose utilization, which becomes pathological through progression into older age. In separate analyses of previously published maternal plasma cortisol levels and csf CRF elevations after VFD, we found positive correlations with juvenile offspring BMI levels in both sexes but more evident among males (15). Furthermore, this percentage elevation in maternal csf CRF was positively correlated with insulin resistance (HOMA) in the young offspring. Interestingly, offspring GLP-1 levels were positively related to maternal subordinance (D.K., M.A.B., I.S., E.L.P.S., J.D.C., L.A.R., J.G.K., unpublished data), supporting the concept that GLP-1 is an early stress-linked response. We conclude that our validated experimental model of stress-related to adverse rearing predicts the early appearance of obesity and insulin resistance.

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REFERENCES

1. Van Itallie TB: Stress: a risk factor for serious illness. *Metabolism* 51:40–45, 2002
2. Clark PM: Programming of the hypothalamo-pituitary-adrenal axis and the fetal origins of adult disease hypothesis. *Eur J Pediatr* 157 (Suppl. 1):S7–S10, 1998
3. Rosenblum LA, Smiley J: Weight gain in bonnet and pigtail macaques: therapeutic effects of an imposed foraging task in disturbed monkeys. *J Child Psychol Psychiatry* 25:485–497, 1984
4. Andrews MW, Rosenblum LA: The development of affiliative and agonistic social patterns in differentially reared monkeys. *Child Dev* 65:1398–1404, 1994
5. Coplan JD, Andrews MW, Rosenblum LA, Owens MJ, Friedman S, Gorman JM, Nemeroff CB: Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders. *Proc Natl Acad Sci U S A* 93:1619–1623, 1996
6. Smith EL, Coplan JD, Trost RC, Scharf BA, Rosenblum LA: Neurobiological alterations in adult nonhuman primates exposed to unpredictable early rearing: relevance to posttraumatic stress disorder. *Ann N Y Acad Sci* 821:545–548, 1997
7. Coplan JD, Trost RC, Owens MJ, Cooper TB, Gorman JM, Nemeroff CB, Rosenblum LA: Cerebrospinal fluid concentrations of somatostatin and biogenic amines in grown primates reared by mothers exposed to manipulated foraging conditions. *Arch Gen Psychiatry* 55:473–477, 1998
8. Coplan JD, Smith EL, Trost RC, Scharf BA, Altemus M, Bjornson L, Owens MJ, Gorman JM, Nemeroff CB, Rosenblum LA: Growth hormone response to clonidine in adversely reared young adult primates: relationship to serial cerebrospinal fluid corticotropin-releasing factor concentrations. *Psychiatry Res* 95:93–102, 2000
9. Smith EL, Batuman OA, Trost RC, Coplan JD, Rosenblum LA: Transforming growth factor beta-1 and cortisol in differentially reared primates. *Brain Behav Immun* 16:140–149, 2002
10. Mathew SJ, Shungu DC, Mao X, Smith EL, Perera GM, Kegeles LS, Perera T, Lisanby SH, Rosenblum LA, Gorman JM, Coplan JD: A magnetic resonance spectroscopic imaging study of adult nonhuman primates exposed to early-life stressors. *Biol Psychiatry* 54:727–735, 2003
11. Kaufman D, Smith EL, Gohil BC, Banerji M, Coplan JD, Kral JG, Rosenblum LA: Early appearance of the metabolic syndrome in socially reared bonnet macaques. *J Clin Endocrinol Metab* 90:404–408, 2005
12. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, Allen K, Lopes M, Savoye M, Morrison J, Sherwin RS, Caprio S: Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med* 350:2362–2374, 2004
13. Cai G, Cole SA, Tejero ME, Proffitt JM, Freeland-Graves JH, Blangero J, Comuzzie AG: Pleiotropic effects of genes for insulin resistance on adiposity in baboons. *Obes Res* 12:1766–1772, 2004
14. Hamelin AM, Habicht JP, Beaudry M: Food insecurity: consequences for the household and broader social implications. *J Nutr* 129 (Suppl. 2):525–528, 1999
15. Coplan JD, Smith ELP, Altemus M, Mathew SJ, Perera T, Kral JG, Gorman JM, Owens MJ, Nemeroff CB, Rosenblum LA: Maternal-infant response to variable foraging demand in nonhuman primates: effects of timing of stressor on cerebrospinal fluid corticotropin-releasing factor and circulating glucocorticoid concentrations. *Ann N Y Acad Sci* 1071:525–533, 2006
16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
17. Jen KL, Hansen BC, Metzger BL: Adiposity, anthropometric measures, and plasma insulin levels of rhesus monkeys. *Int J Obes* 9:213–224, 1985
18. Bodkin NL, Metzger BL, Hansen BC: Hepatic glucose production and insulin sensitivity preceding diabetes in monkeys. *Am J Physiol* 256:E676–E681, 1989
19. Wang Y, Moss J, Thisted R: Predictors of body surface area. *J Clin Anesth* 4:4–10, 1992
20. Fairbanks LA: Early experience and cross-generational continuity of mother-infant contact in vervet monkeys. *Dev Psychobiol* 22:669–681, 1989
21. Kraemer GW: Psychobiology of early social attachment in rhesus monkeys: clinical implications. *Ann N Y Acad Sci* 807:401–418, 1997
22. Clarke AS: Social rearing effects on HPA axis activity over early development and in response to stress in rhesus monkeys. *Dev Psychobiol* 26:433–446, 1993
23. Lubach GR, Coe CL, Ershler WB: Effects of early rearing environment on immune responses of infant rhesus monkeys. *Brain Behav Immun* 9:31–46, 1995
24. Kalin NH, Shelton SE, Davidson RJ: Cerebrospinal fluid corticotropin-releasing hormone levels are elevated in monkeys with patterns of brain activity associated with fearful temperament. *Biol Psychiatry* 47:579–585, 2000
25. Sanchez MM, Hearn EF, Do D, Rilling JK, Herndon JG: Differential rearing affects corpus callosum size and cognitive function of rhesus monkeys. *Brain Res* 812:38–49, 1998
26. Dallman MF, Pecoraro N, Akana SF, La Fleur SE, Gomez F, Houshyar H, Bell ME, Bhatnager S, Laugero KD, Manalo S: Chronic stress and obesity: a new view of "comfort food". *Proc Natl Acad Sci U S A* 100:11696–11701, 2003
27. Hansen BC, Bodkin NL: Beta-cell hyperresponsiveness: earliest event in development of diabetes in monkeys. *Am J Physiol* 259:R612–R617, 1990
28. Kinzig KP, D'Alessio DA, Herman JP, Sakai RR, Vahl TP, Figueiredo HF, Murphy EK, Seeley RJ: CNS glucagon-like peptide-1 receptors mediate

endocrine and anxiety responses to interoceptive and psychogenic stressors. *J Neurosci* 23:6163–6170, 2003
29. Egan JM, Meneilly GS, Habener JF, Elahi D: Glucagon-like peptide-1 augments insulin-mediated glucose uptake in the obese state. *J Clin Endocrinol Metab* 87:3768–3773, 2002
30. Ankel-Simons F: *Primate Anatomy.* 2nd ed. San Diego, CA, Academic Press, 2000, p. 103–106
31. Moran A, Jacobs DR Jr, Steinberger J, Hong CP, Prineas R, Luepker R, Sinaiko AR: Insulin resistance during puberty: results from clamp studies in 357 children. *Diabetes* 48:2039–2044, 1999
32. Qi Y, Takahashi N, Hileman SM, Patel HR, Berg AH, Pajvani UB, Scherer PE, Ahima RS: Adiponectin acts in the brain to decrease body weight. *Nat Med* 10:524–529, 2004
33. Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC, Matsuzawa Y: Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes* 50:1126–1133, 2001
34. Jones AP, Assimon SA, Friedman MI: The effect of diet on food intake and adiposity in rats made obese by gestational undernutrition. *Physiol Behav* 37:381–386, 1986
35. Vadlamudi S, Kalhan SC, Patel MS: Persistence of metabolic consequences in the progeny of rats fed a high-calorie formula in their early postnatal life. *Am J Physiol* 269:731–738, 1995
36. Waterland RA, Garza C: Potential mechanisms of metabolic imprinting that lead to chronic disease. *Am J Clin Nutr* 69:179–197, 1999
37. Levin BE: The obesity epidemic: metabolic imprinting on genetically susceptible neural circuits. *Obes Res* 8:342–347, 2000
38. McEwen BS: Protective and damaging effects of stress mediators. *N Engl J Med* 338:171–179, 1998